



Figure S1: Bioinformatics pipeline for initial classification of germline mutations

Table S1: Pathogenic/likely pathogenic variants

Gene	Mutation	dbSNP	Notes	Sample	VAF Germline	VAF Tumor
APC	NM_001127511:c.806_807insAACAGCC:p.E269fs			IC263	0.25	0.24
BLM	NM_000057:c.3384_3385instATTGTATACTT:p.S1128fs		1	EWS116		0.32
BLM	NM_000057:c.C1933T:p.Q645X	rs373525781	3	NCI0198	0.65	0.51
BRCA1	NM_007300:c.3481_3491del:p.E1161fs	rs80357877		IC315	0.22	0.33
BRIP1	NM_032043:c.C2392T:p.R798X	rs137852986	2	SJEWSS001318	0.67	0.58
ERCC3	NM_000122:c.1421dupA:p.D474fs		1	EWS137		0.47
EXT2	NM_000401:c.69+2insAGGG (splice site)			IC136	0.31	0.21
FANCC	NM_000136:c.C553T:p.R185X	rs121917783	2	SJEWSS001302	0.7	0.58
FANCD2	NM_033084:c.2715+1G>A (splice site)	rs201811817	1	EWS103		0.39
FANCM	NM_020937:c.2191_2192del:p.L731fs			IC349	0.27	0.24
FLCN	NM_144606:c.G918A;p.W306X	rs142934950	1	EWS113		0.55
MITF	NM_000248:c.G952A;p.E318K	rs149617956		IC106	0.36	0.32
PMS2	NM_000535:c.G137T:p.S46I	rs121434629		IC242	0.25	0.33
POLE	NM_006231:c.4090dupC:p.R1364fs			IC275	0.32	0.31
PTCH2	NM_001166292:c.3311_3312insA:p.L1104fs			IC158	0.37	0.35
PTPN11	NM_002834:c.A1529G;p.Q510R	rs121918470		IC054	0.35	0.31
RAD51	NM_001164269:c.G452A;p.R151Q	rs121917739	1	EWS101		0.49
RAD51D	NM_001142571:c.293delA:p.D98fs		1	EWS120		0.76
RET	NM_020630:c.G2370C:p.L790F	rs75030001	2	SJEWSS001308	0.33	0.57
SLX4	NM_032444:c.C5242T:p.Q1748X			IC270	0.29	0.35
TINF2	NM_001099274:c.C936A;p.Y312X	rs201677741		EWS2021	0.52	0.6
TP53	NM_001126115:c.C451T:p.R151C	rs149633775		IC053	0.3	0.4
WRAP53	NM_018081:c.1558dupG:p.C519fs			IC086	0.24	0.33

1=tumor only sequencing

2=previously reported Zheng et al. NEJM 2015

3=previously reported Chang et al. CCR 2016

Table S2: Variants of unknown significance

Gene	Mutation	dbSNP	Notes	Sample	VAF Germline	VAF Tumor
APC	NM_001127510:c.C1240T:p.R414C	rs137854567	1	EWS112		0.51
BRCA2	NM_000059:c.10095_10096insT:p.V3365fs		1	EWS110		0.43
CHEK2	NM_007194:c.C541T:p.R181C	rs137853010		IC111	0.38	0.32
FANCL	NM_001114636:c.1114_1115insATTA:p.T372fs			IC128	0.34	0.33
FH	NM_000143:c.G302A:p.R101Q	rs75086406	2	SJEWS001320	0.33	0.5
FLCN	NM_144997:c.G1333A:p.A445T	rs41419545		IC318	0.33	0.26
HNF1A	NM_000545:c.G92A:p.G31D	rs137853247		IC273	0.35	0.32
MUTYH	NM_001128425:c.1437_1439del:p.479_480del			IC147	0.31	0.31
NBN	NM_002485:c.A511G:p.I171V	rs61754966		IC174	0.35	0.33
RET	NM_020975:c.A2372T:p.Y791F	rs77724903		IC158	0.25	0.34
RNASEL	NM_021133:c.G793T:p.E265X	rs74315364		IC319	0.39	0.37
RNASEL	NM_021133:c.G793T:p.E265X	rs74315364		EWS2004	0.33	0.44

1=tumor only sequencing

2=previously reported Zheng et al. NEJM 2015

Table S3: Heterozygous pathogenic variants in *MUTYH*

Gene	Mutation	dbSNP	Sample	VAF Germline	VAF Tumor
MUTYH	NM_001128425:c.G1187A:p.G396D	rs36053993	IC092	0.35	0.32
MUTYH	NM_001128425:c.G1187A:p.G396D	rs36053993	IC151	0.35	0.4
MUTYH	NM_001128425:c.1147delC:p.L383fs		IC296	0.31	0.34
MUTYH	NM_001128425:c.A536G:p.Y179C	rs34612342	SJEDS1302	0.43	0.59

Table S4: Truncating mutations in non-syndromic tumor suppressor genes

Gene	Mutation	dbSNP	Notes	Sample	VAF Germline	VAF Tumor
DNMT3A	NM_022552:c.G2320T:p.E774X		1	EWS119		0.25
DOCK8	NM_203447:c.54-1G>T (splice site)	rs192864327		EWS2019	0.45	0.42
FOXL2	NM_023067:c.C818A:p.S273X		1	EWS125		0.67
KMT2D	NM_003482:c.C11851T:p.Q3951X		1	EWS120		0.95
KMT2D	NM_003482:c.9272dupC:p.P3091fs		1	EWS129		0.29
PML	NM_033239:c.1998delC:p.I666fs		2	SJEW5001310	0.6	0.36
RNF168	NM_152617:c.C391T:p.R131X	rs201915239	1	EWS107		0.49
RNF43	NM_017763:c.C433T:p.R145X			SJEW5001314	0.44	0.62
TRAF7	NM_032271:c.373delC:p.P125fs		1	NCI0071		1

1=tumor only sequencing

2=previously reported Zheng et al. NEJM 2015

Table S5: Characteristics of Ewing sarcoma patients with relation to presence or absence of germline pathogenic/likely pathogenic mutation

	Germline P/LP mutation		p
	yes	no	
Male	11	74	1
Female	9	56	
Age <12	12	55	0.31
12-24	9	66	
>24	0	9	
Alive	7	55	0.75
DOD	5	29	
Somatic Mutation			
<i>STAG2</i>	4 of 22 (18.1%)	26 of 146 (17.8%)	1
<i>CDKN2A</i>	3 of 22 (13.6%)	18 of 146 (12.3%)	1
<i>TP53</i>	2 of 22 (9.1%)	13 of 146 (8.9%)	1